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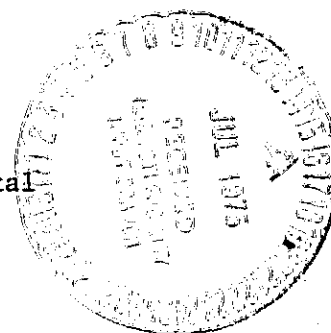
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RENAL EFFECTS OF CONTINUOUS NEGATIVE
PRESSURE BREATHING

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ABSTRACT

Continuous negative pressure breathing (CNPB) was utilized to simulate the thoracic vascular distension of zero G or Space, in 11 anesthetized rats. The animals underwent renal clearance and micropuncture renal nephron studies before, during, and after CNPB. 4 rats were pretreated with a high salt diet and I-M desoxycorticosterone (DOCA) in excess. None of these rats diuresed with CNPB. In contrast 5 of the 7 remaining rats increased the fraction of the filtered sodium excreted (C_{Na}/GFR , $p < .05$) and their urinary flow rate (V , $p < .05$). Potassium excretion increased ($U_K V$, $p < .05$). End proximal tubular fluid specimen's TF/P inulin ratios were unchanged. Whole kidney and single nephron glomerular filtration rates fell 10%.

CNPB, a mechanism for atrial distension, appears to cause, in rat, a decrease in distal tubular sodium, water and potassium reabsorption. Exogenous mineral-corticoid prevents the diuresis, saluresis, and kaluresis.

In a separate group of 5 rats the CNPB was prolonged or excessive (CNPB > 3.5 cm H_2O negative pressure), in these the diuresis ceased and/or antidiuresis occurred. This confirmed the adequacy of other non-atrial volume control mechanisms in regulating renal salt and water conservation in opposition to the studied atrial-renal (Henry-Gauer) reflex of thoracic vascular distension. It encourages us to suggest that the atrial distension reflex diuresis that occurs with shifts of blood to the thorax in assuming a

weightless condition, if comparable to the thoracic vascular distension of this model, will not progressively impair the circulation of the mammal but will be minor and short-lived.

INTRODUCTION

Part of the effect of weightlessness in space is a redistribution of blood volume; in moving from 1 G to 0 G there is a relative blood volume distension of the left atrium. This atrial distension produces a reflex diuresis of the sensed excess volume (Henry-Gauer reflex) (5,7,8). The effector arm and renal mechanism of this reflex are unknown. Our earlier studies of direct left atrial distension in the dog (8) and left atrial stimulation-tachycardia in man (9) are extended here to rat, where the renal effects could be studied more directly. Atrial distension was elicited by allowing the anesthetized rat to spontaneously breathe against a continuous gentle negative pressure (CNPB).

METHODS

16 white male wistar rats were prepared for micropuncture studies of the left kidney in the standard manner (1,12). The rats were anesthetized with inactin intraperitoneally and underwent tracheostomy and jugular vein cutdown. Their kidney was exposed through a flank incision and buffered in a plastic cup under a microscope. In this position the individual superficial cortical nephrons were aspirated for tubular fluid while simultaneous urine production

was collected from the left kidney's cannulated ureter. In addition, occasional 50 microliter blood samples were drawn from the tail vein. These animals received a load of 1-10 ml of normal saline intravenously to replace plasma water losses due to the operation and received sustaining infusions of normal saline with trace carbon-14 labeled inulin at a constant rate of 20 ul/min. The saline load and operation were always completed at least 45 min prior to the beginning of the experiment. Thus, with this preparation one can study mammalian rats' whole kidney clearance function and individual nephron function.

For the experiments reported here a Y-tube was placed over the tracheostomy tube. The second terminus of the tube was allowed to lie open into the ambient air and the third end was placed on-line with a small suction pump. Prior to placement the negative pressure at the Y-tube had been adjusted at the suction pump motor to (-) 1 to (-) 5cm of water.

Preliminary studies with catheterization of the right atrium demonstrated that the change in pressure elicited by CNPB was well transmitted through the airway and across the thin walled low pressure thoracic vasculature.

The animals, with Y-tube in place and suction pump off, were then allowed to equilibrate for at least 45 min. Then 5-12 urine collection periods of 20-45 min each were obtained. During these periods end proximal tubular nephrons were micropunctured and timed volumes of fluid aspirated. The only difference among these urine collection periods was that during the middle 2 to 4 periods the suction pump was on and the animals thorax was subjected to a measured 1 to 5cm

water of negative pressure (C_N). The collection periods prior to turning the pump on are referred to as pre-experimental control periods (C_1), those afterwards as post-experimental control periods (C_2).

The 14 C-inulin activity of all plasma, urine, and tubular fluid samples was counted in a Packard liquid scintillation counter and the CPM adjusted for quenching characteristics in a Wang 720 C computer to yield true DPM. Sodium and potassium in the samples were analyzed with a flame photometer (4). Urine volumes were measured by weight. Tubular fluid volume was calculated in a constant bore capillary tube under a microscope.

Clearance calculations follow accepted formulae (4,8,9):

U_{Na} = (mEq/L) = urinary concentration of sodium

U_K = (mEq/L) = urinary concentration of potassium

V = (ul/min) = urinary flow rate. One kidney

pNa = (mEq/L) = plasma concentration of sodium

C_{Na} = (ul/min) = clearance of sodium. One kidney = $(U_{Na}V)/pNa$

GFR = (ul/min) = glomerular filtration rate. One kidney =

C_{IN} = clearance of inulin = $U \text{ inulin} \times V/P \text{ inulin}$

TF/P inulin = ratio of tubular fluid inulin to plasma

inulin concentration = an index of proximal

tubular nephron fractional reabsorption $(1-P/TF \text{ inulin})$

Distal tubule refers to the nephron beyond the end proximal convoluted tubular micropuncture site and includes the pars recta of the proximal tubule.

Significance was judged on the basis of the t-test at a limit of 95%.

RESULTS

Figure 1 illustrates the best two examples of reactions to CNPB. The broken line (rat 3-16) displays no real change from baseline control values when exposed to CNPB or after its use. This rat was pretreated with DOCA. The opposite effect is seen with the solid line study, illustrating the whole kidney clearance measures in rat 2-24 before, during, and after CNPB. In this animal a reversible increase in fractional salt and water excretion (C_{Na}/GFR and V/GFR) was found associated with CNPB. This represents a decrease in whole kidney sodium and water reabsorption. In addition the absolute excretion of sodium (C_{Na}) and potassium ($U_K V$) rose, and this cation excretion increase was independent of a change in the filtrate concentration of sodium or potassium (pK_a and pK).

Note that in the C_1 control period the DOCA treated rat (3-16) was reabsorbing more sodium (C_{Na}/GFR) than the non-treated animal and that during CNPB potassium excretion in the DOCA treated animal did not rise while a small but significant increase in the non-treated animal did occur.

Recollection end proximal tubular nephron micropuncture samples were obtained in both rats illustrated in Figure 1. The DOCA treated (3-16) rat's mean TF/P inulin was 2.94 before (C_1) and 2.74 during CNPB. The control rat of 2-24 when subjected to negative pressure breathing revealed a mean end proximal TF/P inulin ratio of 2.29, compared to 2.60 during the prior C_1 control period. Thus both animals showed a slight reduction in proximal tubular fractional

sodium and water reabsorption, but an insignificant reduction. In Figure 2 are graphed the recollection pair samples of end proximal tubular fluid TF/P inulin ratios in all the studied rats. The first collection (abscissa) appears to be insignificantly higher than the second in most of the pairs, regardless of whether the pairs are control only (C_1-C_1 , C_2-C_2 , C_1-C_2) or whether the first (C_N-C_2) or second (C_1-C_N) sample is taken during the experimental period of continuous negative pressure breathing.

The same result was found when the micropuncture nephron samples obtained were compared without regard to recollections. 46 control state ($C_{1/2}$) end proximal samples mean TF/P inulin was 2.44. 18 CNPB state samples TF/P inulin was 2.29 (.2pp.1).

Thus in those experiments when CNPB did produce a diuresis, and also in those in which there was no obvious solute diuresis, no evidence of early proximal tubular nephron involvement could be found by recollection micropuncture.

In 5 experiments in which CNPB was excessive in pressure there was a sharp fall in glomerular filtration rate, presumably due to decreased left ventricular filling and decreased cardiac output. With this there was an associated antidiuresis and salt retention. Figure 3 illustrates such a case. None of these 5 animals were included in the results summarized in Figure 2.

DISCUSSION

CNPB was not associated with a diuresis in every case in which it was applied. Reported in Figure 2 are only the 11 cases in which the experimental stimulus was not associated with a precipitous drop in GFR (Figure 3), presumably due to decreased ventricular filling. Even in the 11 cases with a stable GFR the mean filtration rate decreased an average of 10% and a solute diuresis was not always seen. These results are in close agreement with prior human studies of continuous negative pressure breathing where only an occasional marked solute diuresis is found, and the GFR fell in 3 of the 4 reported studies (2). We interpret the lack of uniform effect to suggest that this stimulus (CNPB) has a very narrow range of physiological effectiveness. In some animals at low pressures no effect on renal excretion is found (29% of cases). In other animals at higher negative pressures a moderate impairment of renal function and a resultant antidiuresis and salt retention are found with the fall in GFR (as in Figure 3). Between these two extremes is the normal functioning range of the atrial renal or Henry-Gauer reflex (Figure 1). Thus it is that an occasional human with CNPB will increase sodium excretion 50% or 122 uEq/min, or another patient increase solute excretion from 4.4 to 6.5 cc/min, and with both of these increase urine flows (2), while other human studies show no such effect. As with our rats when CNPB causes a diuresis in humans (2,6,11) the increase in V is associated or starts with an increase in solute (NaCl) excretion.

One explanation for the solute diuresis might be that an in-apparent small change in filtration rate is leading to a slight increase in filtered load of solute and water and urine flow (11). But the opposite was apparent in many of our micropuncture studies, and in prior reports (2): filtration decreased while excretion increased.

It would appear that the solute diuresis occasionally seen, although relatively slight with the stimulus of CNPB in the rat, might represent a real decrease in nephron reabsorption of salt and water - comparable to that suggested by other maneuvers that distend or stimulate the atria (9). If so it is instructive to consider the nephron site of this change in normal salt and water reabsorption. The normal micropuncture results reported here suggest that the site is distal to the convoluted proximal tubule.

The lack of a diuresis during DOCA administration (Figure 1) could be explained by: #1: an insignificant stimulus of CNPB, comparable to other non-DOCA studies. In this case with repeated DOCA treated rat studies a diuresis should be seen. We could not demonstrate it; #2: an inhibition by CNPB of mineral-corticoid affected late distal nephron sites of reabsorption, with competitive overcoming of this inhibition by DOCA administration in excess; #3: an inhibition by CNPB of early distal tubular sites of sodium reabsorption, prior to the mineral-corticoid affected late distal tubule. Presumably in this case the early distal tubular decrease in reabsorption, e.g. at the ascending limb of the Loop of Henle, will increase solute and water supply to the later nephron where with DOCA in

excess it will be entirely reabsorbed.

In agreement with this 3rd hypothesis is the increase in potassium excretion depicted in Figure 1 in the non treated rat and also found in other atrial stimulated experimental states, e.g. chronic space or O G inhabitation (3). If the effect of CNPB or space were entirely at the late distal tubular sites (Hypothesis 2 above) and due to an anti-mineral-corticoid-like effect, then relative potassium retention, not increased K^+ excretion, should be expected. From what we know of renal NaCl and K^+ reabsorption it would appear that the decreased reabsorption at early distal tubular sites is partially but not completely compensated by NaCl reabsorption at later distal sites associated, at these sites, with an increase in the gradient for potassium diffusion into the tubule and hence increased potassium excretion. The evidence suggests an early distal tubular defect (hypothesis 3) that is minor in extent, and that can be easily overridden by an increase in late distal tubular reabsorption, by supplying mineralocorticoid.

In seeming opposition to this explanation is the known occasional increase in free water excretion with atrial stimulation and CNPB found in other studies (8,11). For if free water (CH_2O) is predominately generated at early distal tubular sites and if reabsorption were to be impaired at these sites, as suggested by the third hypothesis, CH_2O would be expected to decrease. However, an increase in free water excretion with V can be due not only to (a) an increase in early distal tubular reabsorption of sodium but also to (b) changes in antidiuretic hormone and late distal water transport, as

illustrated by our prior dog studies of direct atrial distension with a balloon catheter (8), and to (c) increases in solute excretion, as illustrated by our prior study of atrial stimulation-tachycardia in humans (9). Antidiuretic hormone changes would not explain the current findings of an increase in sodium chloride excretion with CNPB in the rat. Thus we would interpret our rat studies as similar to our human studies (9): the increase in V and any associated CH_2O increase is due to a solute cation diuresis, where the solute (NaCl) is generated by decreased reabsorption in the tubules prior to the very late water transport site. This interpretation is supported by results in rat which are comparable to those in human studies of CNPB (2,6,11).

Presumably, as in the CNPB studies where GFR falls due to decreased ventricular filling and cardiac output (Figure 3), there are a large number of mechanisms operating on the kidney to control salt and water or volume balance in the mammal (7). The operation of any one mechanism, such as CNPB or atrial distension (8) may be easily overcome by other volume control mechanisms, such as the GFR-physical factors-proximal tubule system or the renin-angiotensin-aldosterone system. Hence atrial distension or stimulation may result in a limited volume loss, or occasionally no apparent loss at all. Sodium chloride and water losses due to any one mechanism of volume control, such as the atrial-renal reflex (5), may be overridden by the many other such renal mechanisms available. Multiple volume control mechanisms offset any persistently inappropriate functioning of one mechanism.

In striking contrast to the above conclusion and to the results reported here and cited in many other studies of the atrial-renal reflex are the results of the single space monkey study: Bonny (10). This monkey in less than 9 days of weightlessness lost 20% of its body weight, primarily through increased urinary excretion. The monkey lost almost its entire estimated extracellular fluid volume with this large fluid loss-equivalent to 14 liters in a 70 kgm man. The animal went into shock and died. The increased V was associated with an increase in the central venous pressure (2-3cm H₂O) of the order of magnitude suggested here by CNPB. Why volume control mechanisms other than the atrial-renal reflex did not operate to stop this continued renal volume loss is still a mystery, one which only further primate and human studies may answer.

Potassium excretion may not be as privileged as salt and water or volume. The body may continue to lose potassium at the distal tubule, in response to continued impairment of the early distal tubule, with little means available to it to conserve potassium. Complications of hypokalemia such as arrhythmias may develop and cardiac functional impairment is possible. However the initial cause of any such series of events lies in the kidney and the full understanding of the effects of the kaliuresis and of the volume adjustments by the kidney that result in continued potassium wasting in space must await further studies, hopefully in space itself.

FOOTNOTES
TO PAGE 1

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FIGURE LEGENDS

Figure 1. The effect of CNPB on clearance measures of renal function in a DOCA loaded rat (interrupted lines) and a non-treated rat (continuous lines). C_1 and C_2 are control periods prior to and after the imposition of the negative pressure. V/GFR represents the fraction of the filtered water excreted, an inverse index of water reabsorption. C_{Na}/GFR is a similar measure for NaCl. C_{Na} represents the acute absolute sodium clearance by the kidney. U_KV is absolute potassium excretion.

Figure 2. A comparison of micropuncture end proximal tubular fluid samples obtained twice from the same nephron and nephron site. C_1 and C_2 are the pre and post-experimental control periods. C_N represents TF samples taken during the stimulus of negative pressure (CNPB). TF/P inulin here is an index of proximal tubular isotonic salt and water fractional reabsorption.

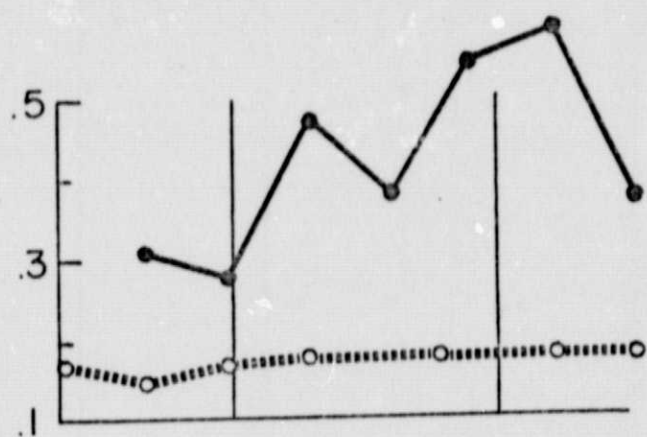
Figure 3. The effect of CNPB at a pressure greater than 5 cm H_2O , negative. Glomerular filtration rate (GFR), urinary flow rate (V), and NaCl clearance (C_{Na}), all fall precipitously with CNPB and return to normal values with the discontinuance of CNPB (C_2).

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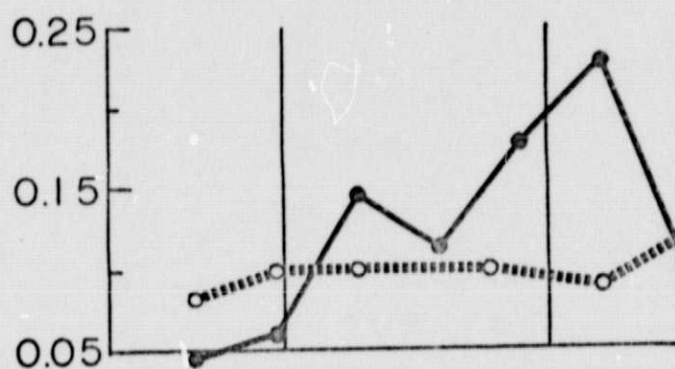
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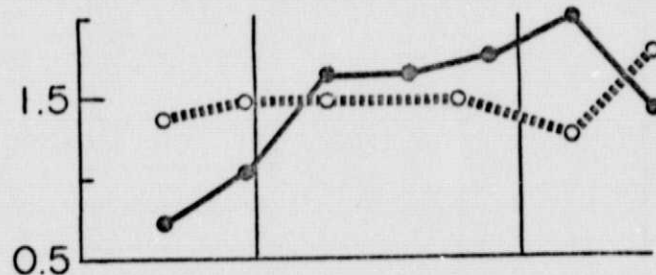
V/GFR (%)



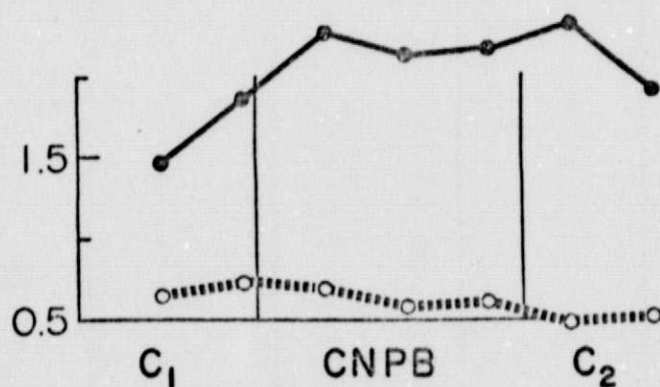
C_{Na} /GFR (%)



C_{Na} (cc/min.)



$U_K V$ (μ Eq/min.)

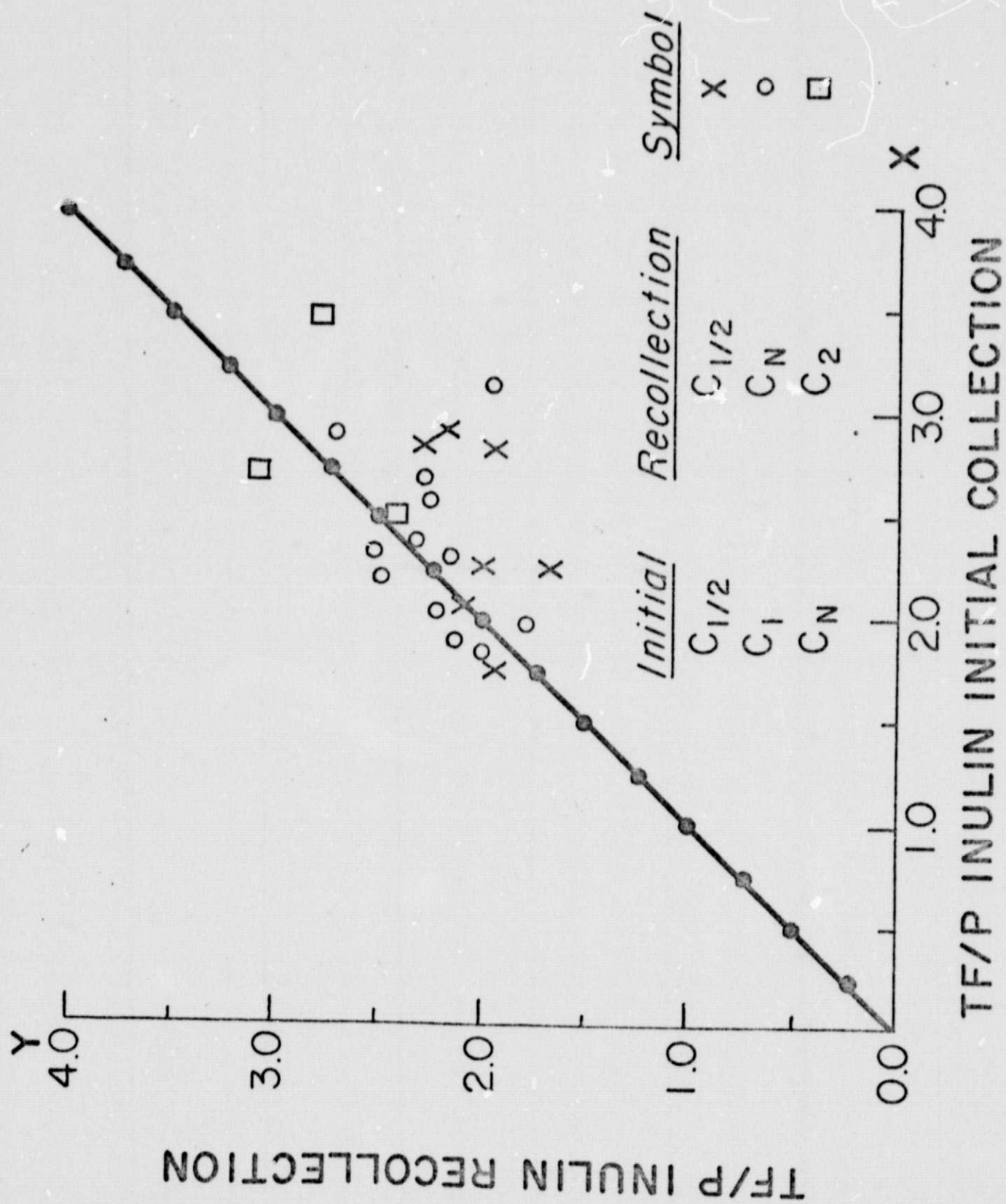


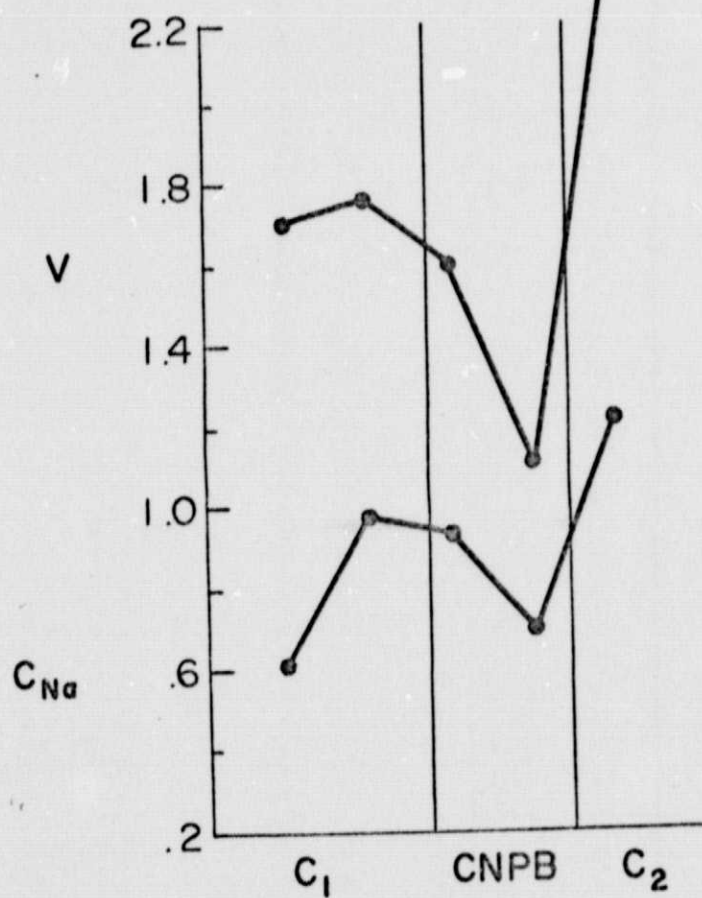
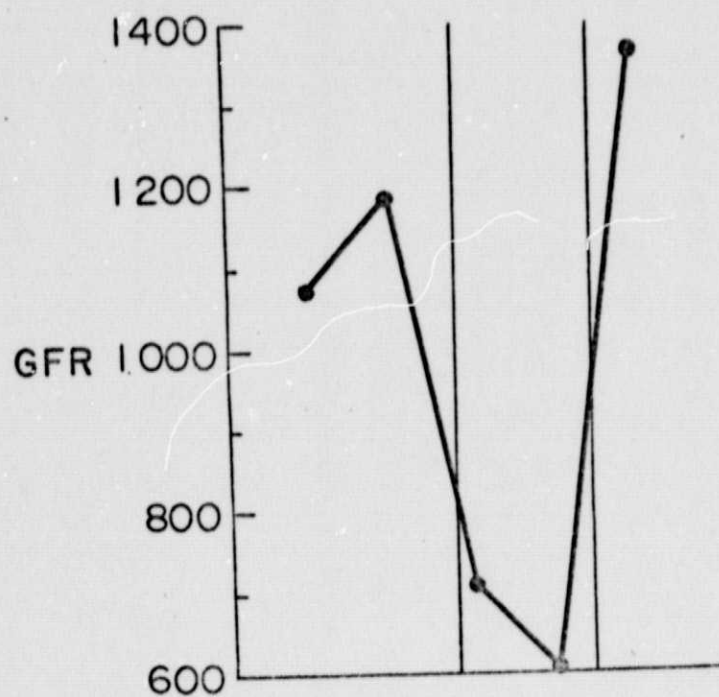
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